

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(Patent Law Article 12, Implementing Regulation No. 56)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT-NU-006	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).	
International application No. PCT/JP03/02394	International filing date 28 February 2003	Priority Date 04 March 2002
International Patent Classification (IPC) or national classification and IPC Int. Cl.⁷ C07K14/80, 7/06, 7/08, 1/12, 1/16; A61K38/17, A61P3/00, 7/00, 9/00, 43/00		
Applicant NIHON UNIVERSITY		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.																
2.	<p>This REPORT consists of a total of 4 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 4 sheet(s).</p>																
3.	<p>This report contains indications relating to the following items:</p> <table style="width: 100%;"> <tr> <td style="width: 5%;">I</td> <td><input checked="" type="checkbox"/> Basis of the report</td> </tr> <tr> <td>II</td> <td><input type="checkbox"/> Priority</td> </tr> <tr> <td>III</td> <td><input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td>IV</td> <td><input type="checkbox"/> Lack of unity of invention</td> </tr> <tr> <td>V</td> <td><input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td>VI</td> <td><input type="checkbox"/> Certain documents cited</td> </tr> <tr> <td>VII</td> <td><input type="checkbox"/> Certain defects in the international application</td> </tr> <tr> <td>VIII</td> <td><input type="checkbox"/> Certain observations on the international application</td> </tr> </table>	I	<input checked="" type="checkbox"/> Basis of the report	II	<input type="checkbox"/> Priority	III	<input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	IV	<input type="checkbox"/> Lack of unity of invention	V	<input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	VI	<input type="checkbox"/> Certain documents cited	VII	<input type="checkbox"/> Certain defects in the international application	VIII	<input type="checkbox"/> Certain observations on the international application
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Date of submission of the demand 07 May 2003	Date of completion of this report 14 January 2004	
Name and mailing address of the IPEA/JP JAPAN PATENT OFFICE KASUMIGASEKI 3-4-3, CHIYODA-KU, TOKYO, JAPAN Postal Code 100-8915	Patent Office Examiner (Authorized Officer)	4N
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I. Basis of the report**1. With regard to the elements of the international application:***

- ☐ the international application as originally filed
- ☒ the description, pages 1-11, as originally filed,
pages , filed with the demand,
pages , received on with the letter of
- ☒ the claims, pages 1-3, as originally filed,
pages , as amended under PCT Article 19
pages , filed with the demand,,
pages 4-22, received on 25 November, 2003 with the letter of
- ☐ the drawings, pages , as originally filed
pages , filed with the demand,
pages , received on with the letter of
- ☒ the sequence listing part of the description
pages 1/5-5/5, as originally filed,
pages , filed with the demand
pages , received on with the letter of

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language which is:

- ☐ the language of a translation furnished for the purposes of international search (under PCT Rule 23.1(b)).
- ☐ the language of publication of the international application (under PCT Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under PCT Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

- ☐ Contained in the international application in printed form.
- ☒ Filed together with the international application in computer readable form.
- ☐ Furnished subsequently to this Authority in written form.
- ☐ Furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (PCT Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under PCT Article 14 (Patent Law Article 14) are referred to in this opinion as "originally filed" (PCT Rules 70.16, 70.17)

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

V. Reasoned statement under PCT Article 35(2) (Patent Law Article 12) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. STATEMENT**

Novelty (N)	Claims 1-22	YES
	Claims	NO
Inventive step (IS)	Claims 18-22	YES
	Claims 1-17	NO
Industrial applicability (IA)	Claims 1-22	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS (Rule 70.7)

Document 1: Samya Othman et al., "Resonance Raman investigation of lysine and N-acetylmethionine complexes of ferric and ferrous microperoxidase", European Biophysics Journal, 1999, Vol. 28(1), pp. 12-25; see the entire document.

Document 2: Jinn-Shyan Wang et al., "Temperature- and pH-dependent Changes in the Coordination Sphere of the Heme c Group in the Model Peroxidase Na-Acetyl Microperoxidase-8", The Journal of Biological Chemistry, 1992, Vol. 267(22), pp. 15310-15318; see the entire document.

Document 3: Shyamalava Mazumdar et al., "Stability and Characterization of Iron(III) and Iron(II) Heme Peptides Encapsulated in Aqueous Detergent Micelles: ¹H NMR and UV-Vis Spectroscopic Studies", Inorganic Chemistry, 1991, Vol. 30(4), pp. 700-705; see the entire document, in particular, Fig. 1.

Document 4: Seiji Yamada et al., "Characterization and Amino Acid Sequences of Cytochromes c₆ from Two Strains of the Green Alga *Chlorella vulgaris*", Bioscience Biotechnology and Biochemistry, 2000, Vol. 64(3), p.628-632, see the entire document.

Document 5: Richard E. Dickerson et al., "Ferricytochrome c", The Journal of Biological Chemistry, 1971, Vol. 246(5), pp. 1511-1535; see the entire document, in particular, Fig. 1 and Appendix.

Document 6: Seiji Yamada et al., "Structure of cytochrome c₆ from the red alga *Porphyra yezoensis* at 1.57Å resolution", Acta Crystallographica Section D; Biological Crystallography, 2000, Vol. D56(12), pp. 1577-1582; see the entire document, in particular, Figure 4.

Document 7: "Capture of Nitrogen Monoxide (NO) by Heme Proteins", Chemistry and Organisms, 1996, Vol. 34(12), pp. 784-785; see the entire document.

Document 1 discloses a heme peptide in which the heme c is coordinated with porphyrin and MP8 peptide. This document also discloses that such a heme peptide is stabilized when the His of MP8-derived Cys-Ala-Gln-Cys-His peptide is linked to the heme c.

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box [V]

Document 2 discloses that MP-8 is an octapeptide consisting of Cys-Ala-Gln-Cys-His-Thr-Val-Glu which is obtained by degradation of cytochrome c, and that MP-8 forms via His one of the six ligands of an iron complex.

Document 3 discloses that trypsin treatment of cytochrome c yields a heme octapeptide consisting of Cys-Ala-Gln-Cys-His-Thr-Val-Glu, and that chymotrypsin treatment of cytochrome c yields a heme undecapeptide consisting of Val-Glu-Lys-Cys-Ala-Gln-Cys-His-Thr-Val-Glu.

Document 4 discloses that the heme-binding domain of cytochrome c has a motif -CXXCH-.

Document 5 discloses a cytochrome c derived from horse.

Document 6 discloses a cytochrome c derived from red alga *Prophyra yezoensis*.

Document 1 discloses an iron complex having MP8 and porphyrin as its ligands; and Documents 1-2 disclose that this complex is stabilized when MP8 is coordinated via its histidine. Therefore, one of ordinary skill in the art could have easily thought of obtaining an iron complex having MP8 and porphyrin as its ligands.

Besides, prior to the priority date of the present application, it was known that treatment of cytochrome c with trypsin or chymotrypsin yields a peptide having Cys-Ala-Gln-Cys-His (as disclosed in Document 3) and that a sequence consisting of -CXXCH- is a heme-binding motif (as disclosed in Document 4). Thus, one of ordinary skill in the art could have easily obtained a heme peptide consisting of a peptide comprising a -CXXCH- motif, such as Cys-Ala-Gln-Cys-His, by treating cytochrome c with a restriction enzyme such as trypsin and purifying the digest by chromatography, etc. Selecting appropriate methods from conventional purification methods in order to improve the purity of the protein of interest is within the scope of routine practice for one of ordinary skill in the art. Further, use of a cytochrome c derived from horse or red alga *Prophyra yezoensis* (such as disclosed in Documents 5-6) as a starting material is also within the scope of routine practice for one of ordinary skill in the art.

Therefore, it is deemed that one of ordinary skill in the art could have easily thought of claims 1-17 based on Documents 1 and 5.

Document 7 discloses that cytochrome c, which is a heme protein, acquires a still higher NO-scavenging ability through denaturation.

Respective inventions according to claims 18-22 are not disclosed in any of the reports cited in the International Search Report and, thus, they are novel. Although Document 7 discloses that cytochrome c, which is a heme protein, acquires a still higher NO-scavenging ability through denaturation, it does not disclose that a heme peptide has NO-scavenging effect. Even one of ordinary skill in the art could not have easily thought of this.